Trail-Following Behavior of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae): Is There a Species-Specific Response?

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Environ. Entomol. 30(3): 457–465 (2001)

ABSTRACT Bioassays were conducted to examine the response of *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) to whole body extracts of termites. Bioassays were also conducted to determine if trail-following behavior could be elicited in glass tubes after different lengths of exposure to termites and if termites showed any species-specific response to exposed tubes. Trail-following behavior was elicited in both species in response to whole body extracts of their nestmates. Although *C. formosanus* responded to the *R. flavipes* extract, *R. flavipes* did not show a difference in response to the *C. formosanus* extract versus solvent-treated controls. Trail-following behavior was elicited in both *C. formosanus* and *R. flavipes* by glass tubes exposed to termites for 5 min. Although neither species showed a preference for glass tubes exposed to their nestmates over tubes exposed to termites of the other species, there were differences in the persistency of trail-following substances deposited in tubes by the two species. When tubes were exposed to termites for 5 min, trail-following behavior was elicited by tubes exposed to *C. formosanus* for at least 1 h after exposure, whereas termites no longer responded to tubes that were exposed to *R. flavipes* 10–15 min after exposure. When tubes were exposed to termites for 14 d, termites deposited chemical markers which lasted for at least 8 d. There was no difference in the response of *C. formosanus* to tubes exposed to their nestmates versus tubes exposed to *R. flavipes.* However, *R. flavipes* showed a significant preference for tubes exposed to their nestmates over tubes exposed to *C. formosanus.* Because there was no evidence of a species-specific response by *R. flavipes* to tubes exposed to termites for only 5 min, it is possible that chemicals in the feces or in salivary secretions deposited in tubes influenced the behavior of *R. flavipes* in tests using tubes exposed to termites for 14 d.

KEY WORDS *Coptotermes formosanus*, *Reticulitermes flavipes*, trail pheromone, chemical communication

SUBTERRANEAN TERMITES READILY follow trail pheromones. The compound (Z,Z,E)-3, 6, 8-dodecatrien-1-ol has been isolated and identified from whole body extracts of *Reticulitermes virginicus* (Banks) and *Coptotermes formosanus* Shiraki, suggesting that it is the trail pheromone of these species of Rhinotermitidae (Matsumura et al. 1968, 1969; Tokoro et al. 1992). This compound has also been isolated and identified from wood decayed by the brown rot fungus, *Gloeophyllum trabeum* (pers. Ex Fr.) Murr (Smythe et al. 1967, Matsumura et al. 1968). Wood decayed by *G. trabeum* elicits trail-following and aggregation behavior in *Reticulitermes* spp. (Esenther and Beal 1979, Grace 1991, Rust et al. 1996) as do other fungal extracts (Grace and Wilcox 1988). Workers of *R. flavipes* (Kollar) orient shelter tubes toward wood blocks decayed by brown rot fungi, *G. trabeum* and *Poria incrassata* (Berkeley & Curtis) Burt (Amburgey and Smythe 1977). Although (Z,Z,E)-3, 6, 8-dodecatrien-1-ol has been isolated from whole body extracts of several species of termites, it has not been collected directly from trails deposited by termites. Also, other nonpheromone chemicals, such as 2-phenoxyethanol, elicit trail-following behavior in both *Reticulitermes* spp. and *C. formosanus* (Chen et al. 1998). Therefore, the identity of the trail pheromone of *C. formosanus* and *R. flavipes* has not been unequivocally established.

In bioassays comparing the trail-following behavior of four subterranean termites species, *R. virginicus*, *R. flavipes*, *R. tibialis* Banks, and *C. formosanus*, all species, except for *R. virginicus*, were able to discriminate between their own extracts and fungal extracts, indicating that there may be additional chemicals contributing to the trail pheromone of these species (Howard et al. 1976). Tokoro et al. (1994) reported the presence of (*Z,E,E*) dodecatrien-1-ol in workers of *C. formosanus* but not in *R. speratus* (Kolbe) and suggested that this compound could be a species-specific factor. Therefore, although (Z,Z,E)-3, 6, 8-dodecatrien-1-ol may be the principal component of the trail pheromone of several species of Rhinotermitidae, there may be secondary components that act as species-specific factors.

Behavioral studies with several species of termites have indicated that trail pheromones are composed of an ephemeral, volatile component and a persistent, nonvolatile component (Traniello and Robson 1995). A volatile component of the trail pheromone of two

other termite species, *Trinervitermes bettonianus* (Sjöst) and *Nasutitermes costalis* (Holmgren), stimulated termites to leave the nest (Oloo and Leuthold 1979, Traniello 1982). In these two termite species, the volatile component elicited recruitment behavior and the persistent component elicited orientation behavior. In choice tests to compare the strength and persistency of trails of *R. flavipes*, evidence was presented for a multicomponent trail pheromone, containing both a long-lasting and an ephemeral component (Runcie 1987).

This study examined the trail-following behavior of the Formosan subterranean termite, *C. formosanus*, and the eastern subterranean termite, *R. flavipes*. Tests were conducted to address the following five questions: (1) Can the two termite species distinguish between whole body extracts of their nestmates compared with extracts of the other species? (2) Can the two termite species distinguish between tubes exposed to their nestmates compared with tubes exposed to the other species after short-term exposure (5 min) of tubes to termites? (3) Can the two termite species distinguish between tubes exposed to their nestmates compared with tubes exposed to the other species after long-term exposure (at least 7 d) of tubes to termites? (4) How persistent are the compounds that elicit trail-following behavior after short-term and long-term exposure to termites? (5) Are there any differences in the persistency of chemical markers deposited in tubes leading to vials containing food compared with tubes leading to vials without food?

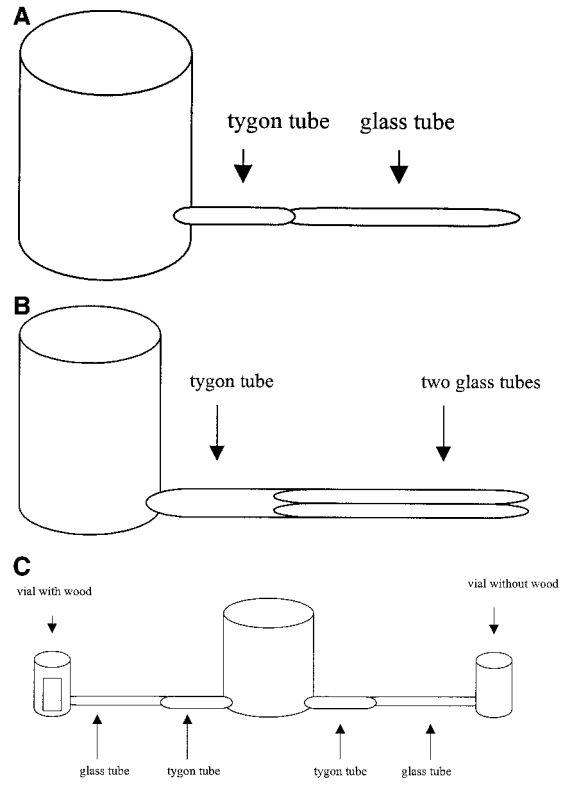

Fig. 1. Testing devices used in short- and long-term exposure tests with glass tubes. (A) Device used to expose glass tubes in short-term exposure tests. (B) Device used in both short- and long-term exposure tests, using an independent group of termites, to test the response of termites to exposed glass tubes in choice test. (C) Device used to expose glass tubes in long-term exposure tests.

Materials and Methods

Termite Collections and Maintenance. Termites were collected from field colonies in New Orleans, LA, using underground bucket traps (Su and Scheffrahn 1986) baited with blocks of spruce, *Picea* sp., wood, and corrugated cardboard rolls. Termites were kept in the laboratory at 22–24°C in 5.6-liter covered plastic boxes containing moist sand, blocks of spruce wood, or cardboard rolls until they were used in experiments. *C. formosanus* and *R. flavipes* were identified to species by using identification keys for soldiers (Scheffrahn and Su 1994). Voucher specimens of soldiers of each colony are stored in 70% alcohol at the Southern Regional Research Center, New Orleans, LA.

Experimental Design. Groups of 200 termites were placed in clear polystyrene, cylindrical screwtop containers (9 cm high by 7 cm diameter). Groups of termites were composed of workers (undifferentiated pseudergates of at least the third instar) and soldiers in a similar caste proportion as occurs in the field (*C. formosanus:* 180 workers, 20 soldiers; *R. flavipes:* 196 workers, four soldiers). In each container, there was 50 g sand (Standard Sand and Silica Company, Davenport, FL), moistened with 10 ml distilled water, and a block of spruce wood (4 by 3.5 by 1 cm) on top of the sand. Each container had a 5-cm length of tygon tubing (0.8 cm diameter) inserted through a hole on one side near the bottom of the container, sealed in place with a glue gun, and capped with a small plastic cap. Termites were able to move freely from the container into the tubing. Glass tubes were connected to the ends of the tygon tubing without disturbing termites in the container (Fig. 1A).

Y-Tube Tests with Whole Body Extracts. Whole body extracts of *C. formosanus* and *R. flavipes* were made by soaking termites in dichloromethane for 30 min. The extract was applied to the stem and one arm of a glass Y-tube (stem: 5.5 cm; arms: 5.0 cm; diameter: 0.6 cm), using a syringe, and the solvent alone was applied to the other arm. The position of the extract and solvent-treated arms on the Y-tube was rotated between replicates to preclude any positional effects. The extract was applied at concentrations of 0.4 worker equivalents/cm and 0.04 worker equivalents/cm because our preliminary results indicated that 0.04 worker equivalents/cm was the minimum concentration at which *C. formosanus* responded to its own extract in these bioassays. Termite responses to extracts of *C. formosanus* and *R. flavipes* were compared directly by applying the extract of *C. formosanus* to one arm and the extract of *R. flavipes* to the other arm of each Y-tube. For all experiments, after the

solvent evaporated, a glass Y-tube was attached to a container as described previously and a single termite was allowed to enter the tube and choose an arm of the Y-tube. If more than one termite entered the tube at the same time, only the choice of the lead termite was counted. In this way, each Y-tube and each termite was only used once to preclude any effects from trail reinforcement or behavioral conditioning. Whole body extracts were made using termites from a single colony of each termite species. For intraspecific tests, extracts were always tested using termites from the same colony as those that were used to make the extract.

The following six tests were conducted at both concentrations: (1) the response of *C formosanus* to an extract of *C. formosanus* versus the solvent alone; (2) the response of *C. formosanus* to an extract of *R. flavipes* versus the solvent alone; (3) the response of *R. flavipes* to an extract of *R. flavipes* versus the solvent alone; (4) the response of *R. flavipes* to an extract of *C. formosanus* versus the solvent alone; (5) the response of *C. formosanus* to an extract of *C. formosanus* versus an extract of *R. flavipes;* (6) the response of *R. flavipes* to an extract of *C. formosanus* versus an extract of *R. flavipes.*

Short-Term Exposure Tests with Glass Tubes. Glass tubes (11.5 cm long by 0.8 cm diameter) were made by breaking off the tips of glass Pasteur pipettes so that termites were able to travel through both ends of the tubes. The broken ends of tubes were heated with a Bunsen burner to smooth edges.

Glass tubes were attached to containers as described previously (Fig. 1A). Bioassays were performed in which termites were allowed to explore glass tubes for 5 min. After 5 min, the glass tubes were disconnected and termites were removed. Each tube was marked on the outer surface on the top of the tube with a permanent marker so that tubes would have the same orientation in bioassays (any "trail" would be located on the bottom of the tube). Each exposed glass tube was paired with a clean glass tube by placing the two glass tubes side by side inside a larger 5-cm length of tygon tubing (1.4 cm diameter). Slits were made on the sides at one end of the tygon tube so that the ends of two glass tubes would fit inside of the tube. The other end of the tygon tube was connected to a cylindrical screwtop container (9 cm high by 7 cm diameter) containing an independent group of ≈200 termites and moist filter paper on the bottom. The opposite ends of the glass tubes extended beyond the tygon tube and were left open (Fig. 1B). For each species, termites were collected from three different colonies. For intraspecific tests, responses to exposed tubes were always tested using termites from the same colony as those that initially explored the tubes.

When termites reached the end of the tygon tubing, they encountered the two glass tubes. The glass tubes were positioned so that termites could not go around them and a small piece of cotton was placed beneath the tubes so that termites could not go under them. Hence, termites had to walk down one of the glass tubes to continue moving forward. The position of exposed and unexposed tubes was rotated between replicates to preclude any positional effects. For each replicate, the first termite to enter one of the glass tubes and travel all the way to the end of the tube was recorded. If more than one termite entered the tube at the same time, only the choice of the lead termite was counted. Each termite and each pair of glass tubes were used only once to preclude any effects from trail reinforcement or behavioral conditioning.

The following eight tests were performed in which termites were allowed to explore glass tubes for 5 min, after which the exposed tubes were left empty for specific lengths of time before testing: (1) responses of *C. formosanus* to tubes exposed to nestmates versus clean tubes; (2) responses of *R. flavipes* to tubes exposed to nestmates versus clean tubes; (3) responses of *C. formosanus* to tubes exposed to *R. flavipes* versus clean tubes; (4) responses of *R. flavipes* to tubes exposed to *C. formosanus* versus clean tubes; (5) responses of *C. formosanus* to tubes exposed to nestmates versus tubes exposed to *R. flavipes;* (6) responses of *R. flavipes* to tubes exposed to nestmates versus tubes exposed to *C. formosanus.* In addition, tests were conducted to determine if increasing the length of exposure of termites to glass tubes would affect the strength and persistency of the trail-following substance; (7) tubes were exposed to *C. formosanus* for 24 h and then paired with tubes that were exposed to *C. formosanus* for 5 min; (8) tubes were exposed to *C. formosanus* for 24 h, left empty for another 24 h, and then paired with unexposed tubes.

Bioassays were also performed where specific numbers of termites were placed in glass tubes for 5 min. For both termite species, tubes exposed to a single termite were paired with clean tubes and tubes exposed to 20 termites were paired with tubes exposed to only a single termite.

Long-Term Exposure Tests with Glass Tubes. For these experiments, 200 termites were placed in containers as described previously, except that each container had two holes with a piece of tygon tubing inserted into each hole. Containers were placed in an unlit environmental chamber at 28°C and 97% RH for 7 d so that termites would become established and begin feeding on the wooden block. After 7 d, the tygon tubes were uncapped and a glass tube was attached to the ends of each tygon tube. The other end of each glass tube was inserted through a hole into a plastic snap cap vial (2.5 cm diameter by 5 cm high). One vial contained 2 g moist sand and a small block (2 by 2 by 0.5 cm) of spruce wood and the other vial contained only moist sand (Fig. 1C). Termites were allowed to move freely between the container and the vials for 14 d. After 14 d, glass tubes were disconnected, and termites were removed. Each tube was marked with a permanent marker on the outer surface on the top of the tube so that tubes would have the same orientation in bioassays (any "trail" would be located on the bottom of the tube). Each glass tube was tested in a paired choice test with a clean glass tube as described previously (Fig. 1B). Tubes were tested after being left empty for 24 h, 1 wk, and 1 mo.

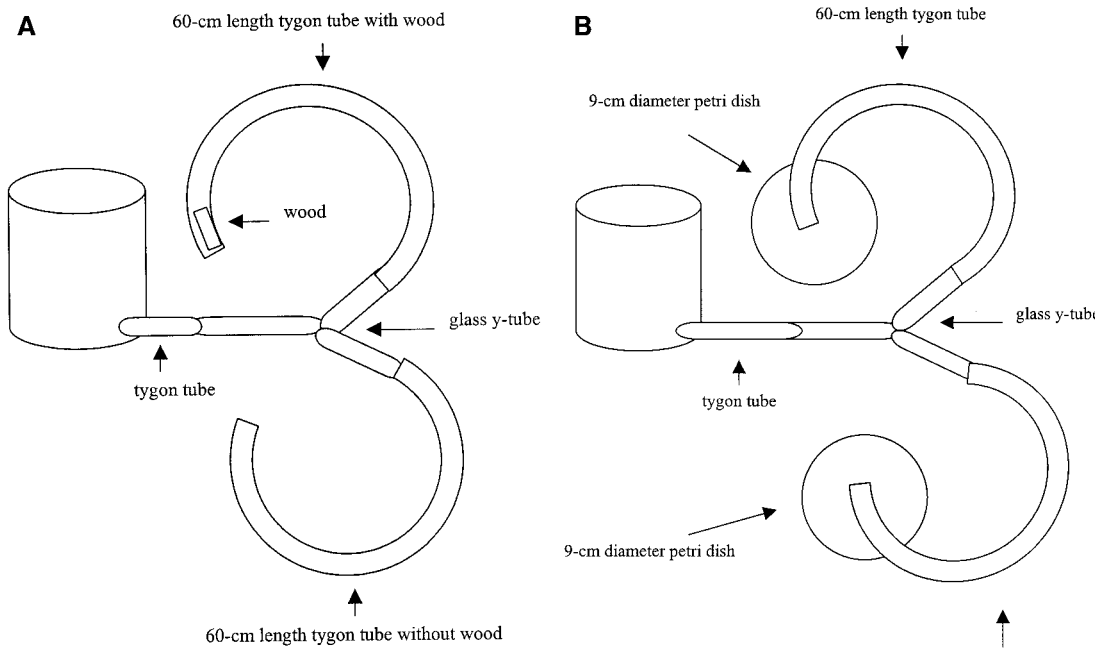

Fig. 2. Testing device used in long-term exposure tests with tygon tubes. (A) Device used to expose 60-cm lengths of tygon tubing. (B) Device, using an independent group of termites, to test the response of termites exposed to 60-cm lengths of tygon tubing.

For each experiment, termites were collected from four different colonies, with five replicates per colony. For intraspecific tests, responses to exposed tubes were always tested using termites from the same colony as those that initially explored the tubes.

Tests were also performed where tubes leading to a vial containing wood were exposed to each termite species for 14 d, and then paired directly with each other to determine whether there was any species-specific response to exposed tubes.

Long-term Exposure Tests with Tygon Tubes. For these experiments, 200 *C. formosanus* were placed in each container as described previously. A glass Y-tube was attached to the end of the piece of tygon tubing attached to each container and a 60-cm length of tygon tubing was attached to each arm of the glass Y-tube. The end of one 60-cm length of tubing contained four pieces of paper-thin yellow pine, *Pinus ponderosa* Laws, wood (2 cm long by 0.1 cm wide by 0.4 cm high) and the other 60-cm length of tubing was empty (Fig. 2A). The ends of both tubes were capped with a plastic cap. The 60-cm length of tygon tubing was curved inward so that the entire testing apparatus could be placed in an unlit environmental chamber at 28°C and 97% RH for 7 d. After 7 d, the number of termites in each tube was determined, and then the glass Y-tubes and the connecting 60-cm lengths of tubing were detached from containers, uncapped, and all termites and wood were removed. Tubes were left empty for 24 h to allow any volatile components to dissipate. After 24 h, the glass Y-tube was attached to a 5-cm length of tygon tubing connected to a container, containing 50 g sand, moistened with 10 ml distilled water, and a new group of 200 *C. formosanus*. A 5-cm length at the end of each tygon tube was cut to eliminate the part of the tube that had contained the wood. The ends of the tubes that had been empty were also cut so that the lengths of all tubes were the same. Then, the end of each tygon tube was placed through a hole in the cover of a polystyrene 9-cm-diameter petri dish so that the end of the tube curved downward into the dish (Fig. 2B). The new group of termites was allowed to move freely from the container into the glass Y-tube and then continue to explore the two arms of tygon tubing. If termites reached the end of the tygon tubing, they would fall into the petri dish and remain in the bottom of the dish. After 1 h, the number of termites in each petri dish was determined.

Bioassays were also conducted where each exposed tygon tube was attached to one arm of a clean glass Y-tube and a clean, unexposed tygon tube of the same length was attached to the other arm of the clean glass Y-tube so that the tube which had contained wood was paired with a clean tube and the tube that had been empty was paired with a clean tube. Even though termites were choosing an arm of a clean glass Y-tube before encountering either tygon tube, termites would often move back and forth within the two arms of a clean glass Y-tube before choosing to move into the tygon tubing. For each experiment, termites were collected from two different colonies of each termite species, with at least four replicates per colony. For tests in which tubes were exposed to *C. formosanus*, responses to exposed tubes were always tested using termites from the same colony as those that initially explored the tubes.

Table 1. Responses of *C. formosanus* and *R. flavipes* to whole body extracts in y-tube tests where one arm of the tube was treated with extract and the other arm was treated with solvent alone

Termite species responding to extracts	Termite species extracted	Concn worker equivalents/cm	Total no. of termites responding	
			Extract-treated	Solvent-treated
C. formosanus	*C. formosanus*	0.40	19	1*
C. formosanus	*R. flavipes*	0.40	16	4*
R. flavipes	*R. flavipes*	0.40	17	3*
R. flavipes	*C. formosanus*	0.40	14	6
C. formosanus	*C. formosanus*	0.04	18	2*
C. formosanus	*R. flavipes*	0.04	15	5*
R. flavipes	*R. flavipes*	0.04	13	7
R. flavipes	*C. formosanus*	0.04	13	7

*, Significant effect of termite responses (sign test; $P \leq 0.05$).

Data Analysis. Statistical analyses of choice tests with glass tubes were based on the binomial distribution, using the Sign test, where results were significant when $P < 0.05$ (SYSTAT 1996). For long-term exposure tests with tygon tubes, the numbers of termites in tygon tubes counted immediately before tests were dismantled and the numbers of termites in petri dishes after 1 h were compared using a *t*-test for matched pairs (SYSTAT 1996).

Results

Y-Tube Tests with Whole Body Extracts. In tests where termites were choosing between extract-treated and solvent-treated arms of a Y-tube, *C. formosanus* showed a significant preference for the extract of its nestmates and for the *R. flavipes* extract over the solvent-treated tubes at both concentrations tested (Table 1). However, *R. flavipes* only showed a significant preference for the extract of its nestmates at a concentration of 0.4 worker equivalents/cm, but not at 0.04 worker equivalents/cm. Also, *R. flavipes* did not show a significant preference for the *C. formosanus* extract over the solvent-treated tubes at either concentration tested (Table 1). In tests where termites were choosing between an extract of their nestmates versus the other species' extract, neither termite species were able to distinguish between their own extract and the other species' extract at either concentration tested (Table 2).

Short-Term Exposure Tests With Glass Tubes. When glass tubes were exposed to termites for 5 min, and then paired with clean, unexposed glass tubes immediately afterward, both species of termites showed a significant preference for tubes exposed to nestmates over unexposed tubes and for tubes exposed to termites of the other species over unexposed tubes (Table 3). Results from bioassays designed to determine the persistency of the compounds that elicited trail-following behavior determined that *C. formosanus* showed a significant preference for tubes exposed to their nestmates over unexposed tubes after exposed tubes had been left empty for up to 1 h. Tubes exposed to *C. formosanus* did not elicit trail-following behavior after being left empty for 2 h. However, *R. flavipes* did not show a significant preference for tubes that had been exposed to their nestmates and left empty for only 10–15 min over unexposed tubes (Table 3). When the responses of both species of termites to tubes exposed to the other species and then left empty for 30 min were tested, *C. formosanus* did not choose tubes exposed to *R. flavipes* over unexposed tubes, whereas *R. flavipes* did show a preference for tubes exposed to *C. formosanus* over unexposed tubes (Table 3). In paired choice tests, neither species showed a preference for tubes exposed to their nestmates over tubes exposed to the other species (Table 4).

Table 2. Responses of *C. formosanus* and *R. flavipes* to whole body extracts in y-tube tests where each arm of the y-tube was treated with an extract of either *C. formosanus* or *R. flavipes*

Termite species responding to extracts	Concn worker equivalents/cm	No. of termites responding to whole body extracts of	
		C. formosanus	*R. flavipes*
C. formosanus	0.40	9	11
R. flavipes	0.40	12	8
C. formosanus	0.04	10	10
R. flavipes	0.04	10	10

Table 3. Responses of *C. formosanus* and *R. flavipes* to glass tubes exposed to termites for 5 min and then tested in paired choice tests against unexposed, clean tubes

Termite Species		Time between initial exposure and test, min[a]	No. of termites responding	
Responding to exposed tubes	Glass tubes exposed to		Exposed tubes	Unexposed tubes
C. formosanus	*C. formosanus*	≤5	18	2*
R. flavipes	*R. flavipes*	≤5	17	3*
R. flavipes	*C. formosanus*	≤5	17	3*
C. formosanus	*R. flavipes*	≤5	16	4*
C. formosanus	*C. formosanus*	30	11	0*
C. formosanus	*C. formosanus*	60	15	5*
C. formosanus	*C. formosanus*	120	13	7
R. flavipes	*R. flavipes*	10–15	14	6
R. flavipes	*R. flavipes*	30	13	7
R. flavipes	*R. flavipes*	60	11	9
R. flavipes	*C. formosanus*	30	17	3*
C. formosanus	*R. flavipes*	30	12	8

*, Significant effect of termite responses (sign test; $P \leq 0.05$).

[a] Tubes were left empty for different lengths of time before being tested.

Table 4. Responses of *C. formosanus* and *R. flavipes* to glass tubes exposed to their nestmates versus glass tubes exposed to termites from the other species in paired choice tests

Termite species responding to exposed tubes	Time between initial exposure and test, min[a]	No. of termites responding to tubes exposed to each species	
		C. formosanus tubes	*R. flavipes* tubes
C. formosanus	≤5	10	10
R. flavipes	≤5	9	11
C. formosanus	10–15	5	5

[a] Tubes were left empty for different lengths of time before being tested.

Coptotermes formosanus chose tubes that had been exposed to nestmates for 24 h over tubes that had been exposed to nestmates for 5 min in only four out of 10 replicates (sign test; $P = 0.75$). Also, *C. formosanus* chose tubes that had been exposed to nestmates for 24 h, and then left empty for another 24 h, over unexposed tubes in only 11 out of 20 replicates (sign test; $P = 0.82$).

When specific numbers of termites were placed in tubes, both *C. formosanus* and *R. flavipes* showed a significant preference for tubes exposed to single termites over unexposed tubes, but did not show a preference for tubes exposed to 20 termites over tubes exposed to only a single termite (Table 5).

Long-Term Exposure Tests with Glass Tubes. In these tests, termites were observed traveling back and forth between the container and both the vial containing wood and the vial without wood throughout the 14-d test. When the response of termites to tubes exposed to members of their own colony for 14 d was tested, *C. formosanus* showed a significant response to tubes leading to wood and to tubes leading to an empty vial compared with unexposed tubes after tubes had been left empty for 24 h and 8 d, but not for 30 d (Table 6). Also, *R. flavipes* showed a significant response to tubes leading to wood compared with unexposed tubes after tubes had been left for 24 h and 8 d, but only showed a significant response to tubes leading to an empty vial after being left empty for 24 h. There was no response by *R. flavipes* to exposed tubes that had been left empty for 30 d (Table 6).

When termites were given a choice between tubes exposed to nestmates for 14 d versus tubes exposed to the other species for 14 d, *C. formosanus* chose tubes exposed to their own colony in only nine out of 20 replicates ($P = 0.82$), but *R. flavipes* chose tubes exposed to their own colony in 16 out of 20 replicates ($P = 0.01$).

Table 5. Responses of *C. formosanus* and *R. flavipes* to glass tubes exposed to different numbers of termites from their own colony for 5 min

Termite species responding to glass tubes	No. of termites exposed to		No. of termites responding to	
	Glass tube 1	Glass tube 2	Glass tube 1	Glass tube 2
C. formosanus	1	0	15	5*
R. flavipes	1	0	15	5*
C. formosanus	20	1	12	8
R. flavipes	20	1	13	7

*, Significant effect of termite responses (sign test; $P \leq 0.05$).

Table 6. Responses of *C. formosanus* and *R. flavipes* to glass tubes exposed to their nestmates for 14 d and then tested in paired choice tests against unexposed, clean tubes

Termite species glass tubes exposed to	Time between exposure to termites and choice test[a]	No. of termites responding to tubes exposed to their nestmates			
		Tubes connected to vial with wood		Tubes connected to vial without wood[b]	
		Exposed tubes	Unexposed tubes	Exposed tubes	Unexposed tubes
C. formosanus	24 h	15	5*	16	3*
C. formosanus	8 d	18	2*	16	3*
C. formosanus	30 d	9	11	10	9
R. flavipes	24 h	18	2*	19	1*
R. flavipes	8 d	16	4*	14	6
R. flavipes	30 d	9	11	14	6

*, Number of termites choosing exposed and unexposed tubes is significantly different (sign test; $P \leq 0.05$).

[a] Tubes were left empty for different lengths of time before being tested.

[b] In tests with *C. formosanus,* there was one replicate where termites never entered the tube connected to the vial without wood because the entrance to the tube was plugged up with sand. Therefore, there were only 19 glass tubes tested.

Long-Term Exposure Tests with Tygon Tubes. In these tests, a large number of termites were observed in tygon tubes containing wood throughout the 7 d. Although large numbers of termites were observed exploring tygon tubes without wood initially, numbers of termites in these tubes declined over time. After 7 d, only small numbers of termites were observed in tygon tubes without wood. In counts made immediately before each replicate was dismantled, mean number of termites in tygon tubes with wood was 39.77 ± 5.8 and mean number of termites in tygon tubes without wood was 5.8 ± 2.4 (Paired *t*-test, $P < 0.001$). In bioassays where tubes had been left empty for 24 h before the glass Y-tube was attached to a container with a new group of termites, the number of *C. formosanus* that traveled to the ends of tubes that had contained wood was greater than the number of *C. formosanus* that traveled to the ends of tubes that had not contained wood in experiments using tubes exposed to either *C. formosanus* or *R. flavipes* (Table 7). In tests where one of the exposed tygon tubes was attached to one arm of a clean glass Y-tube and an unexposed, clean tygon tube was attached to the other arm, more *C. formosanus* traveled to the ends of exposed tubes that had contained wood than to clean tubes and equal numbers of *C. formosanus* traveled to the ends of exposed tubes that had been empty compared with clean tubes (Table 7).

Discussion

Trail-following behavior of *C. formosanus* was elicited by whole body extracts of either species at con-

Table 7. Mean number (±SEM) of *C. formosanus* in petri dishes after 1 h in tests in which tygon tubes were exposed to termites for 14 d

Termite species tubes exposed to:	Tube 1/Tube 2	Replicates	No. of *C. formosanus* in petri dishes connected to tygon tubes[a]	
			Tube 1	Tube 2
C. formosanus	Wood/no wood	9	56.11 ± 18.41a	13.44 ± 5.42b
R. flavipes	Wood/no wood	12	41.91 ± 7.74a	21.91 ± 8.68b
C. formosanus	Wood/clean	9	39.11 ± 9.64a	5.44 ± 2.89b
C. formosanus	No wood/clean	9	15.22 ± 6.23a	11.56 ± 3.24a

Means followed by the same letters within a row are not significantly different (*t*-test for matched pairs; $P \leq 0.05$).

[a] Tubes were left empty for 24 h before being tested. Response of a new group of termites to tubes was determined by connecting the glass y-tube, with tygon tubes attached to both arms, to a new container and placing the end of each tygon tube into a petri dish so that the end of the tube curved downward into the dish. If termites reached the end of the tygon tubing, they would fall into the petri dish and remain in the bottom of the dish.

centrations of 0.40 and 0.04 worker equivalents/cm. In addition, *C. formosanus* did not choose an extract of its nestmates over the extract of *R. flavipes* in a paired choice test. Therefore, there is no evidence that *C. formosanus* can distinguish between an extract of its nestmates over an extract of *R. flavipes.* Because *R. flavipes* did not show a significant response to the *C. formosanus* extract versus the solvent-treated control at 0.40 worker equivalents/cm, but did show a significant response to an extract of its nestmates at that concentration, there could possibly be a species-specific response by *R. flavipes* to whole body extracts. However, there was no difference in the response of *R. flavipes* to an extract of its nestmates versus the *C. formosanus* extract in a paired choice test at either concentration tested. Therefore, the evidence for a species-specific response by *R. flavipes* to whole body extracts is equivocal. The differences in the responses of the two species to extracts of their nestmates at 0.04 worker equivalents/cm could be due to differences in the behavioral response thresholds of the two species or to differences in their behavioral responses to the conditions of the bioassay.

Trail-following behavior was elicited by ephemeral compounds deposited by *C. formosanus* and *R. flavipes* that were allowed to explore glass tubes for 5 min. The presence of the ephemeral substance in tubes did not result in any species-specific response by termites to the trails. Neither species showed a preference for glass tubes exposed to their nestmates over tubes exposed to termites of the other species. Although no differences in the trail-following behavior of the two species were detected, there were differences in the persistency of chemical markers deposited by the two species. Trail-following behavior of both species was elicited by tubes exposed to *C. formosanus* and then left empty for 30 min, whereas neither species responded to tubes that were exposed to *R. flavipes* and then left empty for 30 min. Furthermore, *R. flavipes* did not show a preference for tubes exposed to nestmates and left empty for only 10–15 min compared with unexposed tubes. In contrast, *C. formosanus* showed a significant preference for tubes exposed to nestmates and left empty for 1 h, compared with unexposed tubes. These results suggest that there may be a difference in the concentration of the ephemeral substances deposited by each species. In another study, *R. flavipes* varied the trail they deposited under different circumstances (Runcie 1987). A trail made by displaced workers walking through a tube only lasted for 5 min, and a trail made by a single worker that was allowed to move freely from the nest into a clean tube lasted for 10–15 min (Runcie 1987). Therefore, the difference in the persistency of the trails left by each species may have been caused by differences in their behavioral responses to the conditions of the bioassay.

In short-term exposure tests, trail-following behavior was elicited in response to tubes exposed to only a single termite for 5 min. However, no differences were detected in the response of termites to tubes exposed to 20 termites compared with tubes exposed to only a single termite. A study of the trail-following behavior of *R. hesperus* Banks determined that termites responded to increasing concentrations of sternal gland extract (Grace et al. 1988). However, the difference in the concentration gradient between a single termite and 20 termites placed in tubes for 5 min was not sufficient to elicit a stronger trail-following response in this study.

In bioassays where termites were able to explore empty glass tubes for 24 h, *C. formosanus* did not deposit a persistent trail pheromone. When glass tubes that had been explored by termites for 24 h were paired with tubes explored for only 5 min, there was no difference in the response of *C. formosanus* to tubes. Moreover, glass tubes that had been explored for 24 h and then left empty for another 24 h, did not elicit any trail-following response from termites. In bioassays where termites were allowed to explore glass tubes leading to vials for 14 d, persistent chemical markers were deposited in tubes that lasted for at least 8 d. After 14 d of exposure, termites had deposited feces in tubes and, in many replicates, termites also deposited sand in tubes. Therefore, trail-following behavior could have been elicited by chemicals in feces and salivary secretions, as well as chemicals secreted by sternal glands. Souto and Kitayama (2000) have suggested that chemicals in termite feces are used to maintain established trails of *Constrictotermes cyphergaster* (Silvestri) because these chemicals persist for

months, whereas chemicals secreted by the sternal gland persist for less than 1 h.

In bioassays where termites were presented with a choice of tubes exposed to *C. formosanus* or to *R. flavipes* for 14 d, and then left empty for 24 h, there was no difference in the response of *C. formosanus* to tubes exposed to their nestmates versus tubes exposed to *R. flavipes.* However, *R. flavipes* showed a significant preference for tubes exposed to their nestmates over tubes exposed to *C. formosanus.* These results suggest that a chemical marker deposited in this long-term exposure test was used by *R. flavipes* to distinguish between tubes exposed to the two termite species. Because there was no evidence of a species-specific response by *R. flavipes* to tubes exposed to termites in short-term exposure tests, it is possible that chemicals in the feces or in salivary secretions deposited in tubes influenced the behavior of *R. flavipes* in these tests.

In bioassays where termites were allowed to move freely between a sand-filled container and two 60-cm lengths of tygon tubes connected to arms of a glass Y-tube, numbers of termites in tubes with wood were much greater than numbers of termites in tubes without wood after 7 d, indicating that termites had largely abandoned tygon tubes without wood. When the tubing was left empty for 24 h before being tested, significantly more *C. formosanus* traveled down the exposed tubes that had contained wood compared with the exposed tubes without wood in experiments using tubes exposed to both *C. formosanus* and *R. flavipes.* Also, significantly more *C. formosanus* traveled down exposed tubes that had contained wood compared with clean tubes. In contrast, equal numbers of *C. formosanus* traveled to the end of exposed tubes that had been empty compared with clean tubes. These results suggest that under these conditions, termites deposited chemical markers leading to the wood that were still detectable after 24 h, but did not leave chemical markers that were still detectable after 24 h in the tubes leading to a dead end.

Differences in termite responses to tubes in long-term exposure tests were related to the degree of activity in tubes. In the long-term exposure tests with glass tubes, termites continually traveled back and forth between the container and vials with wood and vials without wood throughout the experiment. Hence, chemical markers in tubes leading to vials with and without wood lasted for at least 24 h for both termite species, and for at least 8 d for *C. formosanus.* In long-term exposure tests with 60-cm lengths of tygon tubes, large numbers of termites moved into tygon tubes containing wood, but termites generally abandoned tygon tubes without wood. Hence, chemical markers lasted for at least 24 h in tubes containing wood, but not in tubes without wood.

In the current study, termites were able to detect the presence of ephemeral substances released from a single termite placed in a glass tube for 5 min. Hence, in these two species, termites seem to leave behind an ephemeral substance wherever they go. Therefore, the ephemeral component of the trail pheromone in these two species may serve as a chemical marker to orient termites toward tunnels where termites are actively foraging. For instance, termites of both species will construct a network of tunnels in sand-filled arenas that do not contain wood, but they will completely abandon those tunnels as soon as food is discovered in another interconnected arena (M.L.C., unpublished data). Ephemeral chemical markers may facilitate shifts in the movement of foragers once food has been discovered. Persistent chemical markers may be used to mark trails leading to food and other well established passageways.

Differences in the persistency of chemical markers in tubes may be due to the deposition of ephemeral and persistent trail pheromone components, differences in the concentration of a single trail pheromone component, chemicals in feces or salivary secretions, or to a combination of these factors. Differences in the responses of the two termite species to whole body extracts and differences in the persistency of trail-following substances deposited in short-term exposure tests may have been caused by differences in the behavioral response thresholds of the two species or in their behavioral responses to the conditions of the bioassay. Although there was no evidence of a species-specific response by *C. formosanus* in any of these bioassays, *R. flavipes* may be able to distinguish trails deposited by its nestmates over trails deposited by *C. formosanus* after long-term exposure.

Acknowledgments

We thank Tina Albrecht for her invaluable technical assistance on this research project. We also thank Ashok Raina, Ken Grace, and Donald Daigle for their valuable comments and suggestions on earlier versions of the manuscript.

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Received for publication 27 July 2000; accepted 2 February 2001.